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Continuous downstream process of monoclonal antibody developed based on the process analysis/understanding and its validation

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Manufacturing Technology Association of Biologics(MAB)

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The project focused on developing the next generation key technologies for manufacturing biologics sponsored by the Ministry of Economy, Trade and Industry (METI), Japan and the Japan Agency for Medical Research & Development(AMED).

Established on September 26, 2013

Corporate members ABLE, ASAHI CHEMICAL, Asahi Kasei Medical, CellFiber, Chitose Laboratory, Chromocenter, Cultivecs, DAIICHI SANKYO, Epistra, FUJIFILM Wako Pure Chemical, FUJIMORI KOGYO, GlycoTechnica, Hitachi, Ina Research, JNC, KANEKA, Kohjin Bio, Kyoto Monotech, Kyowa Kirin, MOCHIDA PHARMACEUTICAL, MORIMOTO-PHARMA, NIKON, Nippon Zenyaku Kogyo, On-chip Biotechnologies, SHIMADZU, Synplogen, Takara Bio, TOKIWA-Bio, Tokyo Chemical Industry, TOKYO KEISO, Toray Industries, U-Medico, ViSpot, YAMASA, YMC, Yokogawa Electric

36 Companies, 5 Universities, 2 National Research Agency, 3 Organizations,
(As of April 4, 2022)
http://cho-mab.or.jp/english/

First-stage 2013-2017
Single-use-technology
Second-stage 2018-2020
Continuous-manufacturing
2018-2023
Gene-cell therapy project
AAV production

How we developed our CDSP
(our mission or my complicated journey)

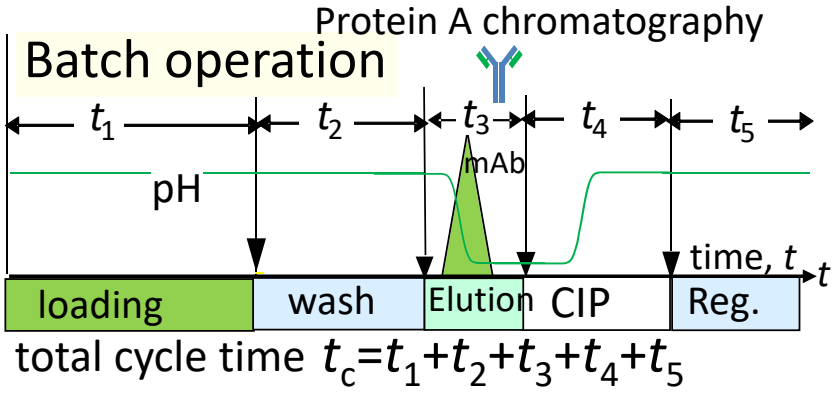
Three year project (2018-2020) for continuous manufacturing of mAb

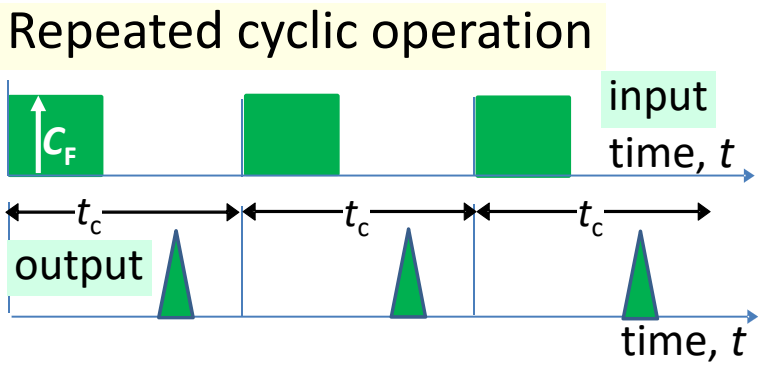
1. Based on our standard platform batch process
   Protein A capture, low pH VI, Polish with 2 columns, VF
2. Process analysis/understanding of each process based on mechanistic models for CDSP
3. Experimental validation (Feed rate = 0.5 to 4 L/d).
   GMP run 10 L/d connected to a perfusion reactor (Goal).

Conversion of batch to continuous

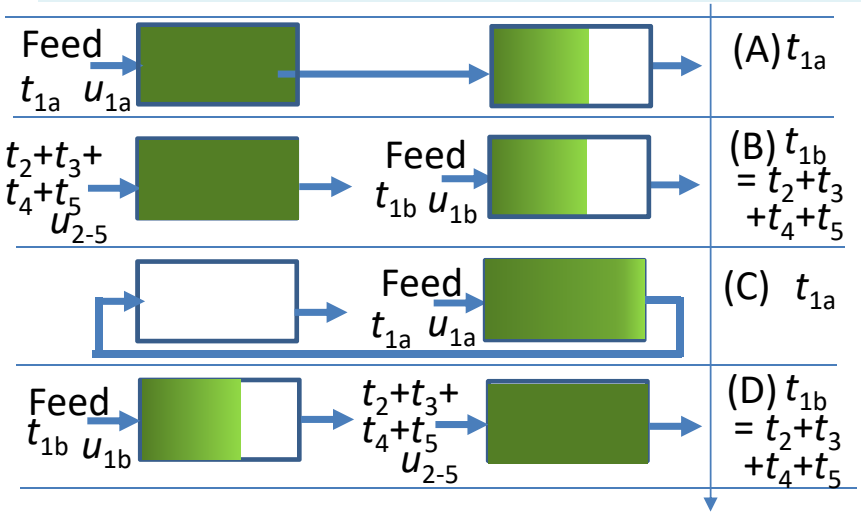
Batch		Continuous
Capture chromatography step PAC (Bind/Elute operation)	[1]	Multi-column periodic counter-current chromatography (PCCC)
Buffer exchange + filtration		Continuous buffer exchange + filtration
Low pH virus inactivation (VI)	[2]	Flow-reactor VI
Buffer exchange + filtration		Continuous buffer exchange + filtration
Polishing chromatography step 1 (Bind/Elute or flow-through operation)	[3]	Flow-through chromatography 1(FTC)
Buffer exchange + filtration		Continuous buffer exchange + filtration
Polishing chromatography step 2 (Bind/Elute or flow-through operation)		Flow-through chromatography 2(FTC)
Virus filtration (constant pressure)		Virus filtration (constant flow)

[1] Continuous capture chromatography
Periodic counter-current operation

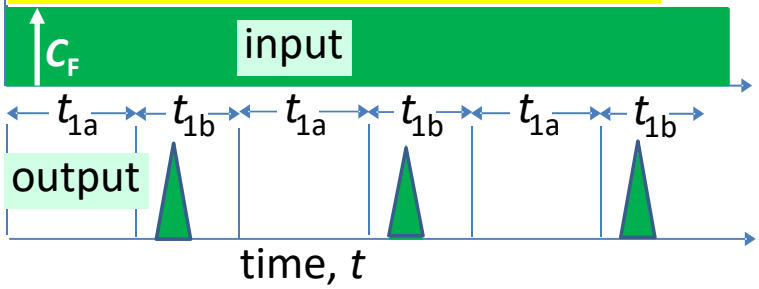




Periodic counter-current chromatography(PCCC) 2-column PCCC



- Loading to connected 2 columns (t_{1a}) and a single column(t_{1b})
- Continuous input, periodic output
- Buffer usage reduction
- 5 to 20 fold concentration



Mechanistic modeling of capture chromatography process optimization

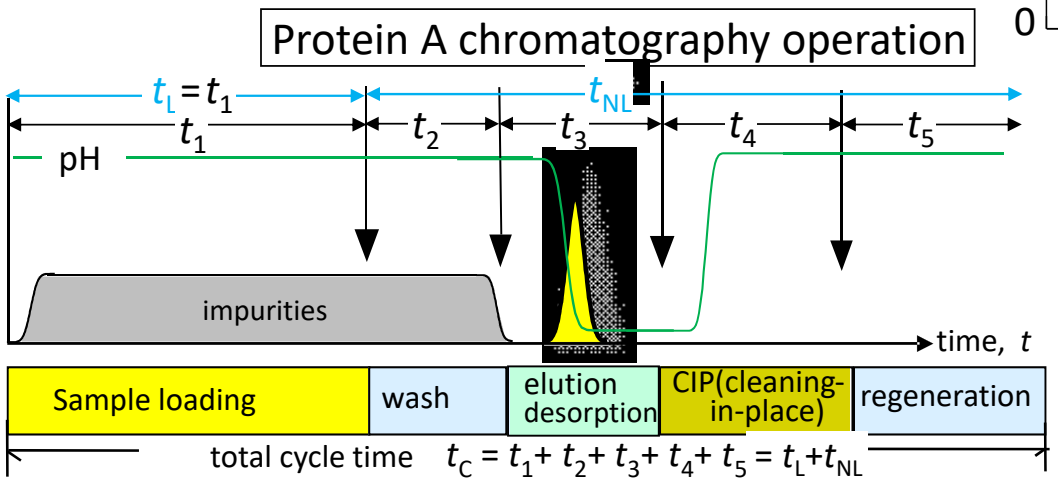
$$\text{Productivity} = \frac{\text{Protein applied}}{(\text{column volume x cycle time})}$$

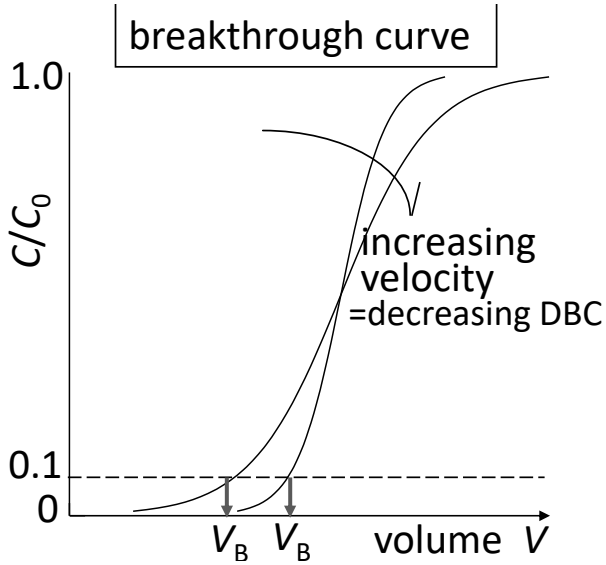
$$P = C_0 t_1 F_v / (V_t t_c) = \text{DBC}/t_c$$

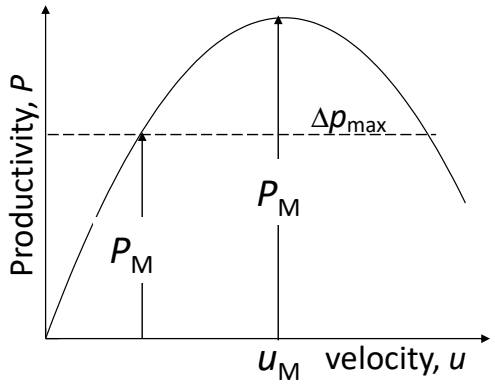
$$\text{DBC} = C_0 V_B / V_t$$

$$t_C = t_1 + t_2 + t_3 + t_4 + t_5 = t_L + t_{NL} = t_{NL} + a(V_t/F_v)$$

P increases when $(d\text{DBC}/du) > 1/(dt_C/du)$

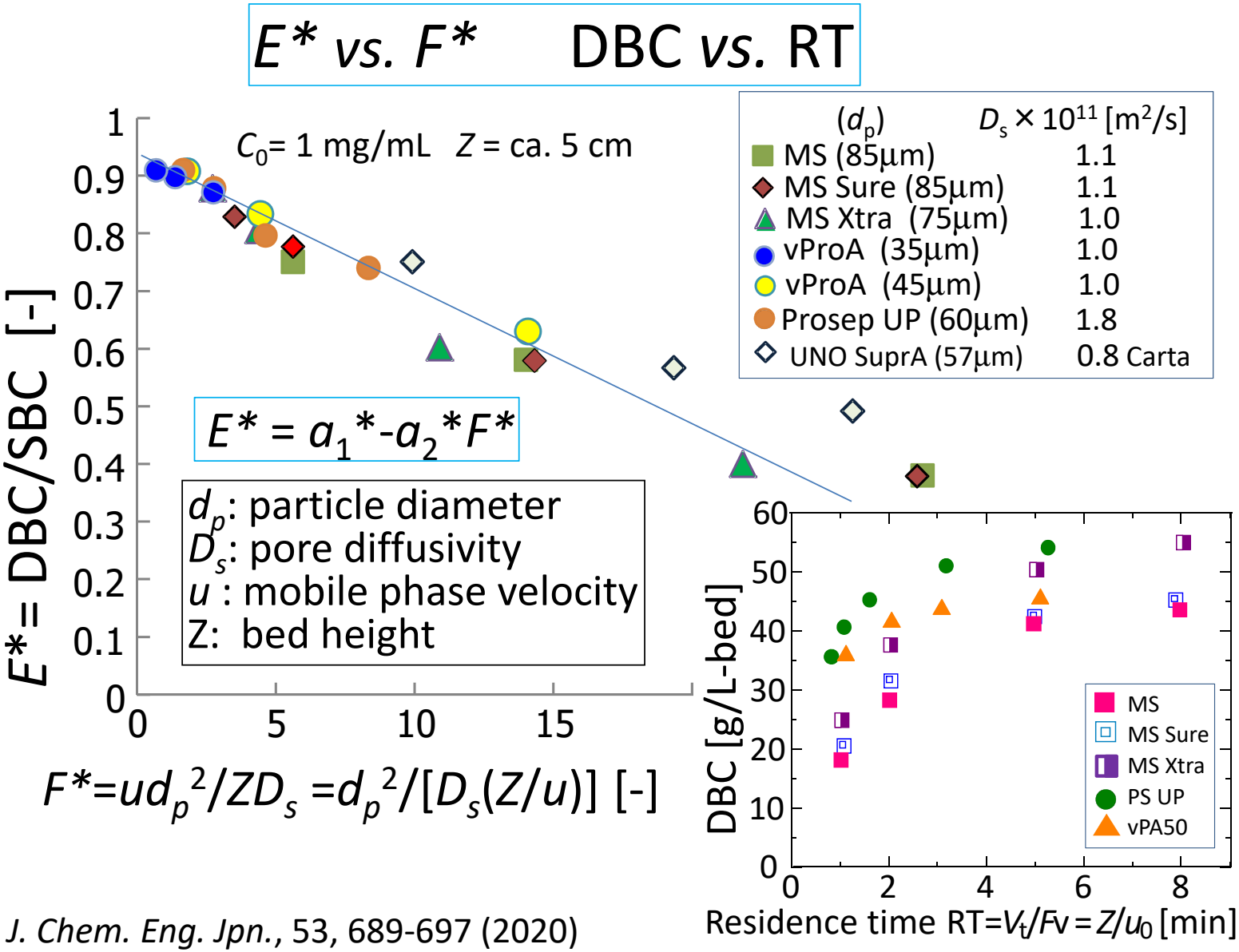






Prediction of the performance of capture chromatography processes of proteins and its application to the repeated cyclic operation optimization. *Journal of Chemical Engineering of Japan*, **53**, 689-697 (2020).

Yamamoto(1992) Short-cut method for predicting the productivity of affinity chromatography

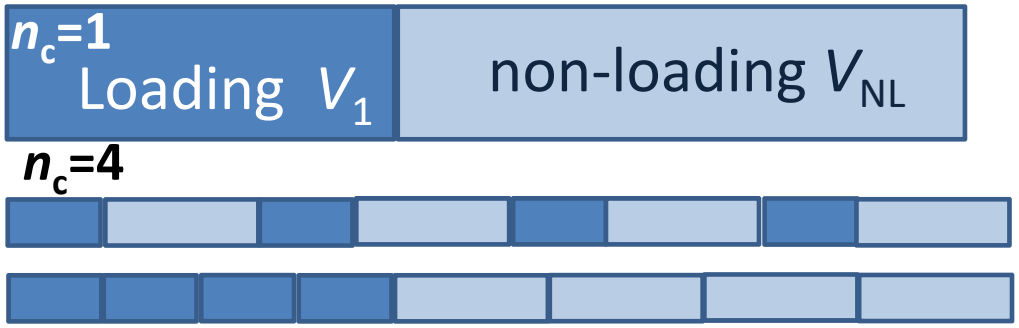


J. Chem. Eng. Jpn., 53, 689-697 (2020)

In the real process, the total process volume V_F (concentration C_F) and the total process time t_{tot} are fixed.
Then, the productivity P is only determined by the bed volume V_t.

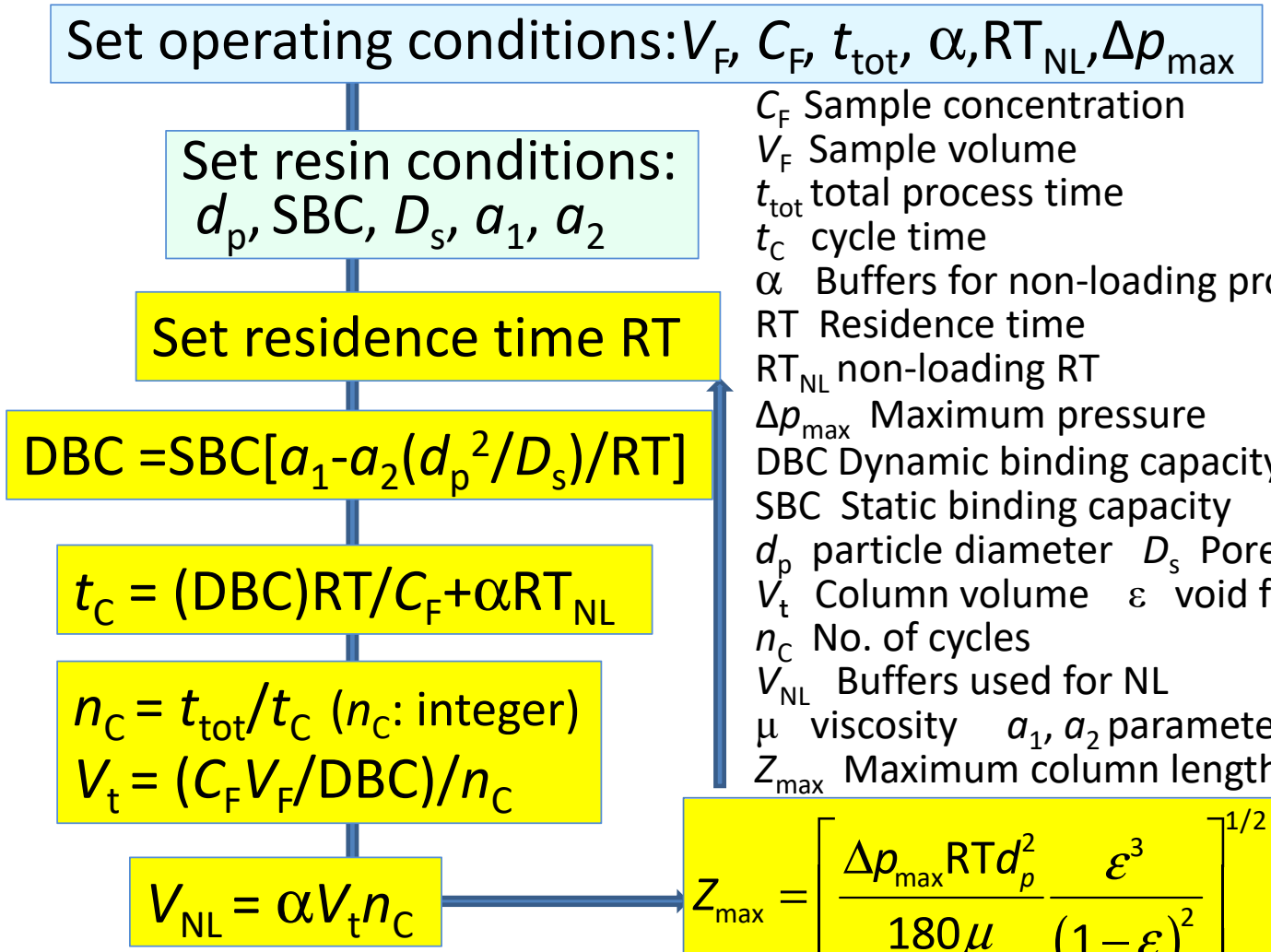
$$P = (C_F V_F)/(t_{tot} V_t)$$

When we perform multiple runs within $t_{tot} = n_C t_C$, the bed volume V_t decreases, which results in a higher P value. (n_C : the number of runs).
By increasing n_C, t_C decreases. DBC also decreases.



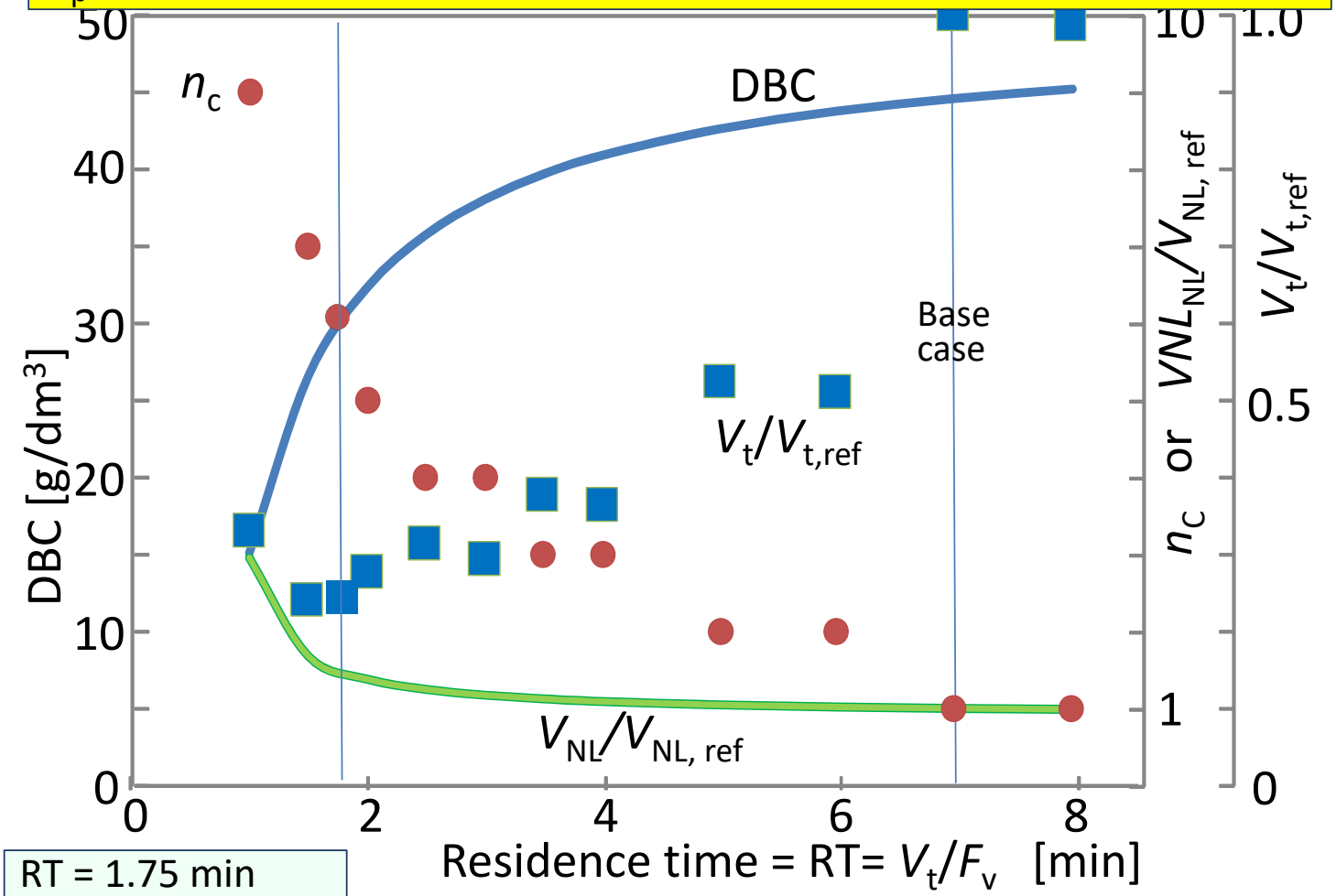
Multiple cycles results in smaller bed volume. However, due to smaller DBC at short residence time, buffer consumption increases.

Calculation procedure $V_F(C_F)$ should be processed within t_{tot}.



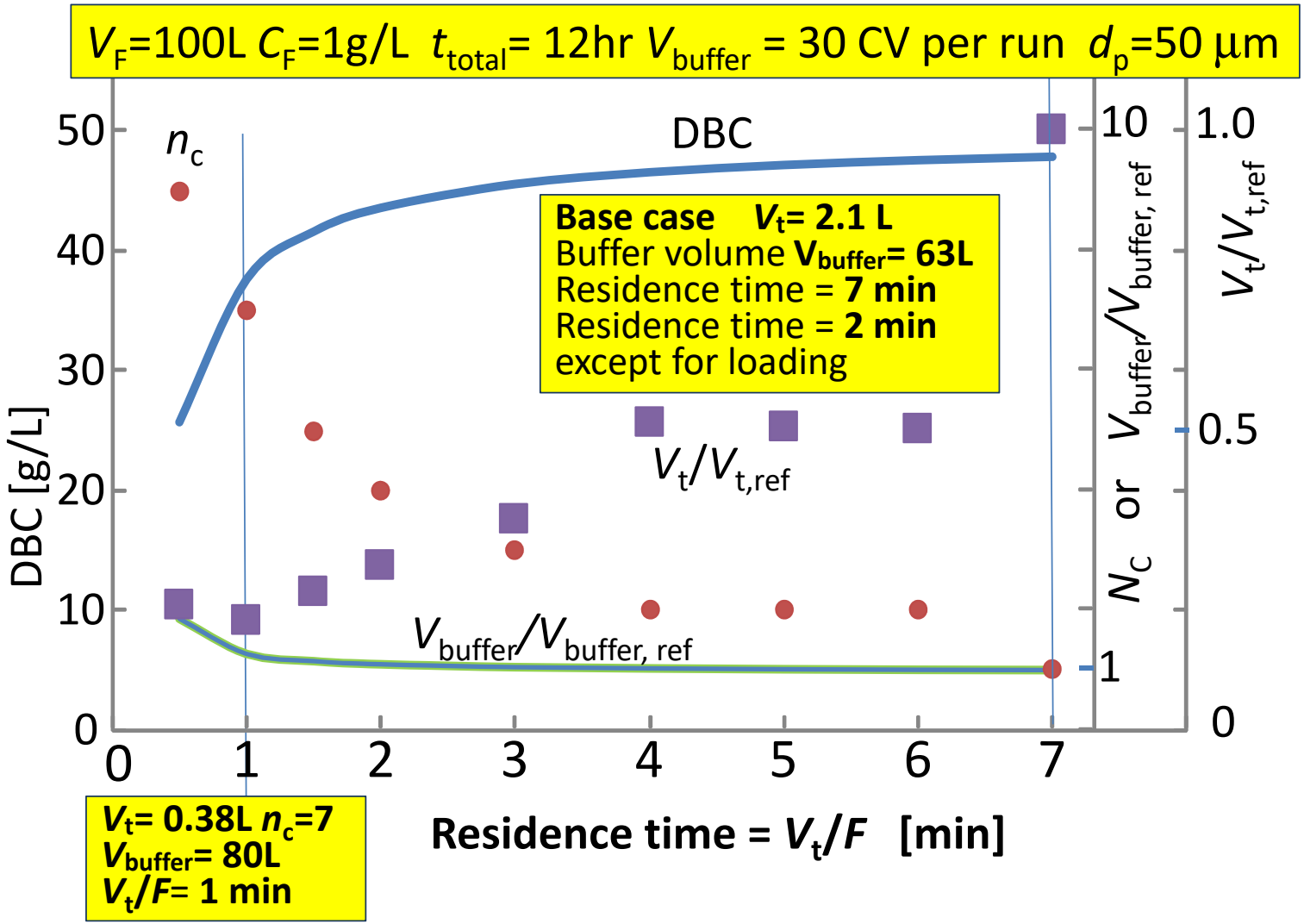
C_F Sample concentration
V_F Sample volume
t_{tot} total process time
t_C cycle time
α Buffers for non-loading protocol(NL)
RT Residence time
RT_{NL} non-loading RT
Δp_{max} Maximum pressure
DBC Dynamic binding capacity
SBC Static binding capacity
d_p particle diameter D_s Pore diffusivity
V_t Column volume ε void fraction
n_C No. of cycles
V_{NL} Buffers used for NL
μ viscosity a_1, a_2 parameters for DBC
Z_{max} Maximum column length

Case A : V_F=100L C_F=1g/L t_{total}= 12hr V_{buffer} = 30 CV per run
d_p=85 μm SBC＝55 g/L



RT = 1.75 min
V_t= 0.56L n_c=6
V_{buffer}= 100L

V_t= 2.2 L nc=1 Buffer volume **V_{buffer}= 66L**
RT = **7 min**, RT_{NL} (for non-loading) = **2 min**



J. Chem. Eng. Jpn., 53, 689-697 (2020)

Table Calculated *P* values for Case A, B and C

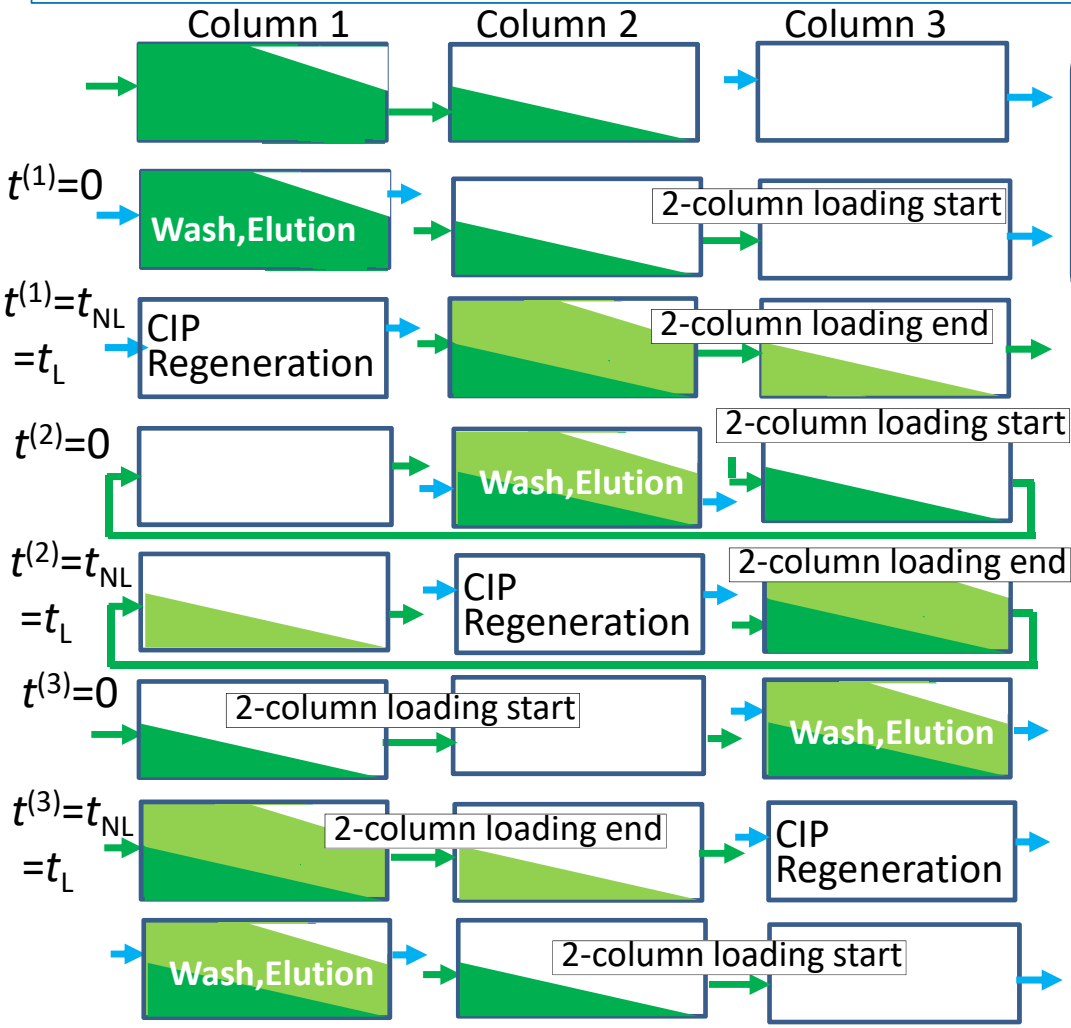
Cell culture supernatant V_F= 100L, C_F= 1g/L t_{total}= 12hr
Base case (single run) V_t = 2.2 L V_{buffer}=66 L RT= 7 min

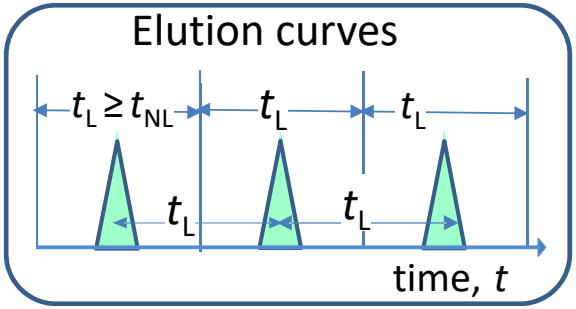
SBC g/L	d_p μm	n_c	RT min	V_t L	V_{NL} [D] L	DBC g/L	t_C min	P g/(h·L)	Z_m [E] cm	E^*
55[A]	85	1	7	2.24	67.3	44.6	372.1	7.2	41.6	0.81
55[A]	85	6	1.75	0.56	101	29.8	112.2	16	20.8	0.54
55[B]	50	7	1	0.38	79.8	37.6	97.6	23.1	9.3	0.68
90[C]	50	5	1	0.33	48.8	61.5	121.5	30.4	9.3	0.68
90[C]	50	7	0.75	0.26	54.5	55.0	101.3	32.6	8.0	0.61

[A] Case A, [B] Case B, [C] Case C
[D] Note that the minimum V_{NL} is given by $\alpha(C_F V_F/\mathrm{SBC})$; 54.5 L for Case A and B, and 33.3 L for Case C.
[E] Bed height due to the pressure limit 0.1 MPa

J. Chem. Eng. Jpn., 53, 689-697 (2020)

The most simple case:3-column PCCC operation



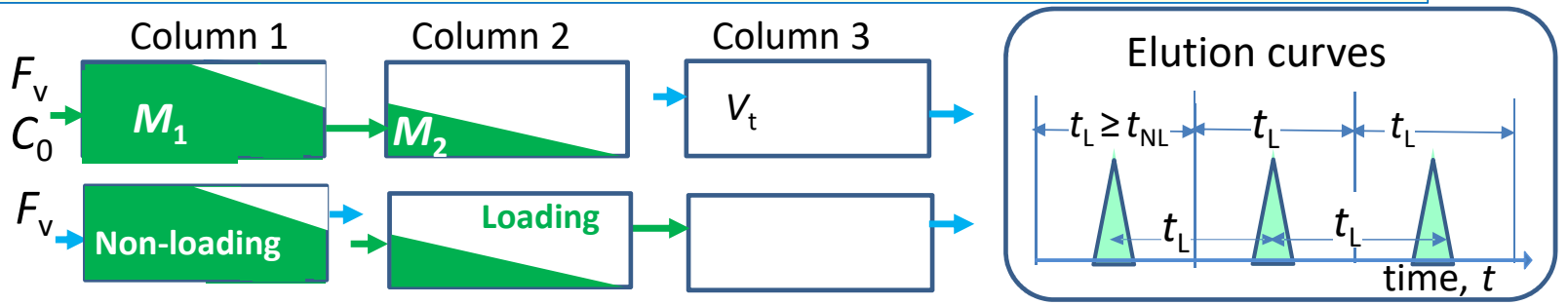


Loading time for the connected 2-columns (tandem column)

$t_L \geq t_{NL}$

Non-loading time (wash, elution, CIP, regeneration)

The most simple case:3-column PCCC operation



	3-column PCCC	Single column batch
1)	$M_{tot}=M_1+M_2=C_0V_B=\mathrm{DBC}(2V_t)$ $\mathrm{DBC}=f(\mathrm{RT}=2V_t/F_V)$	$M_{Batch}=\mathrm{DBC}(V_t)$ $\mathrm{DBC}=f(\mathrm{RT}=V_t/F_V)$
2)	$t_L=(M_1/C_0)/F_v$ $\quad t_L \geq t_{NL}$	$t_L=(M_{Batch}/C_0)/F_v$
3)	$P=M_1/[(t_L)(3V_t)]=C_0F_V/(3V_t)$	$P=M_{Batch}/(t_CV_t)$, $t_C=t_L+t_{NL}$
4)	$V_{NL}^*=V_{NL}/M_1$ $=(V_{NL}/V_t)/(\mathrm{SBC}\cdot E^*)$	$V_{NL}^*=V_{NL}/M_{batch}$ $=(V_{NL}/V_t)/(\mathrm{SBC}\cdot E^*)$

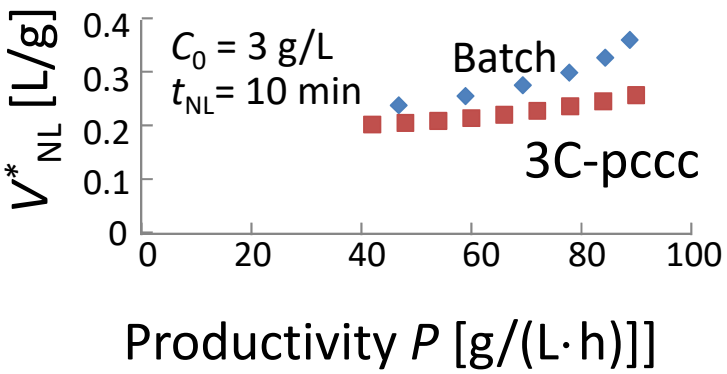
E^*:bed utility, DBC: dynamic binding capacity SBC:static binding capacity
t_L :loading time, P: productivity, V_{NL}=buffer volume for non-loading protocol

A Regressive approach to the design of continuous capture process with multi-column chromatography for monoclonal antibodies

J. Chromatography A, 1658(2021) DOI:10.1016/j.chroma.2021.462604

Buffer consumption V^*_{NL} *vs.* productivity *P*

V_{NL} = 14 CV, $V^*_{NL} = V_{NL}/(C_0 DBC)$, $V^*_{NL,0} = V_{NL}/(C_0 SBC) = 0.204$



-Maximum *P* for batch and 3C-pccc are similar

-Buffer consumption increases with *P*.

-Buffer consumption for 3C-pccc is smaller than batch by 10-40%.

-When t_{NL} is long, maximum *P* for 3C-pcc is smaller than batch because of the constraint $t_L \geq t_{NL}$.

-t_{NL} and V_{NL} are important parameters

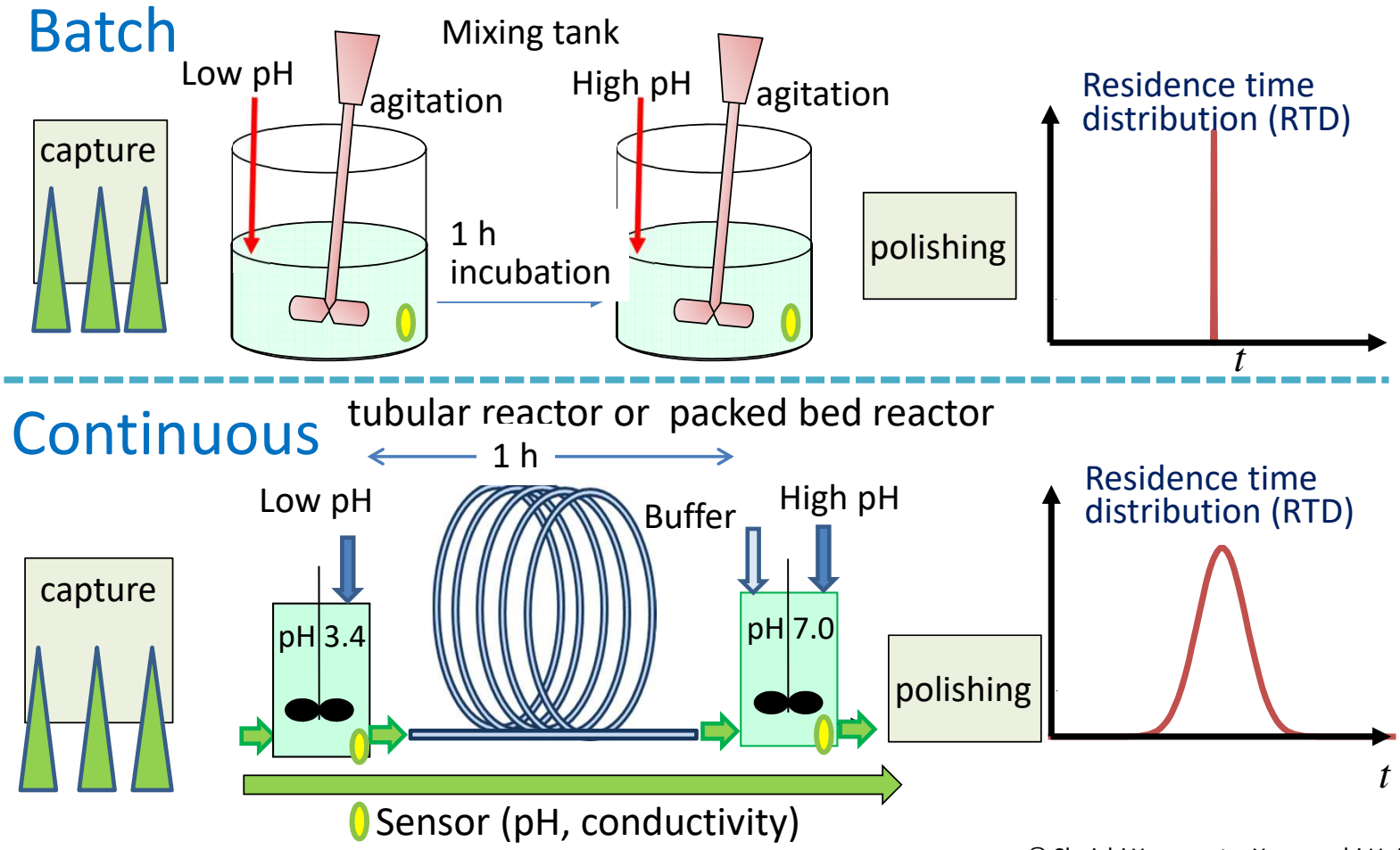
Non-loading protocol in this study

	CV	RT (min)	time (min)
Equilibrium	3	1	3
Post load wash	2	0.5	1
Wash	2	0.5	1
Elution	4	0.5	2
CIP	3	1	3
Total V_{NL}=	14	–	t_{NL}=10

Summary

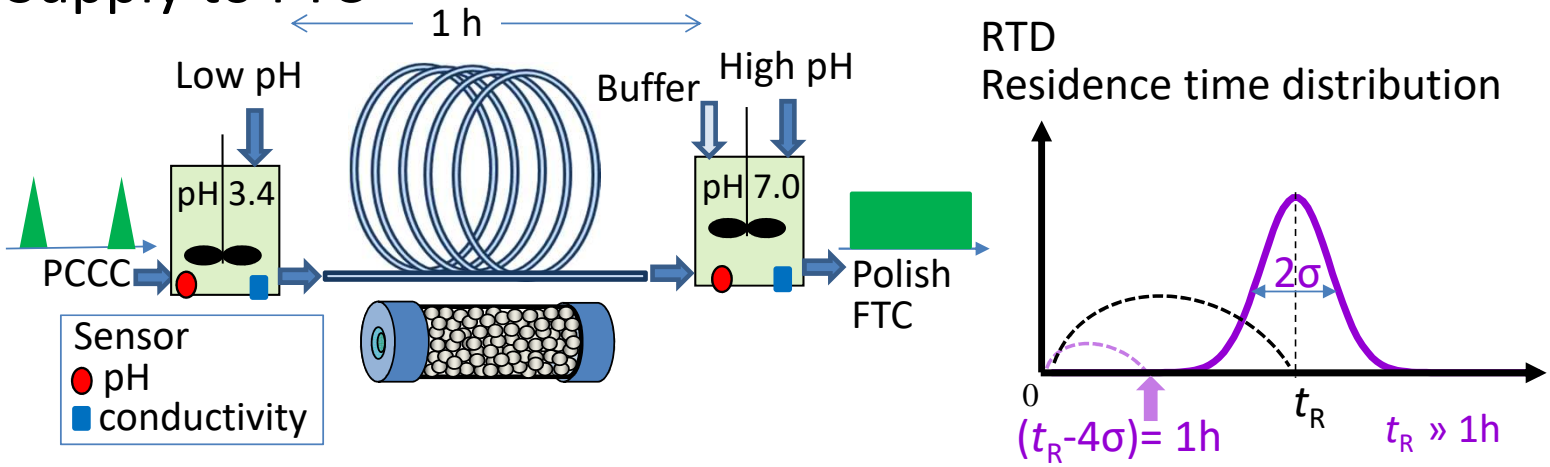
- Productivity can be increased both by continuous or repeated batch operation. Namely, the bed volume can be reduced.
- PCCC can reduce the buffer consumption up to 30-40%.
- PCCC process is strongly influenced by the non-loading protocol.
- As PCCC output is not continuous but intermittent, it is not easily connected to the following virus inactivation reactor.
- As PCCC reduces the volume, the volumetric flow rate is reduced for the following processes. This is important to consider for scale-down studies
- Mechanistic model based analysis is important for the continuous and repeated batch process characterization.
- Optimized repeated batch operation is as efficient as continuous operation.

[2] Continuous low pH virus inactivation by flow reactor
Tubular reactor or packed bed reactor



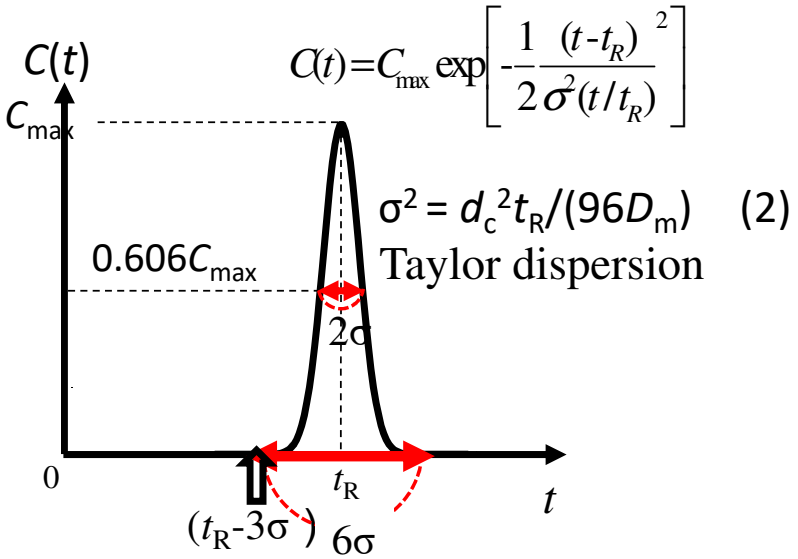
[2] Continuous low pH virus inactivation by flow reactor Tubular reactor or packed bed reactor}

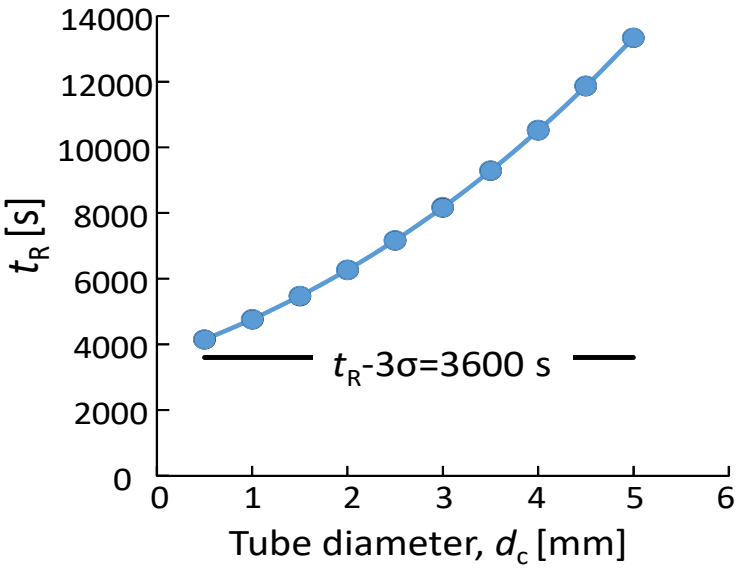
1) Collection of PCCC elution curves into a tank
2) Automated pH adjustment for low pH in a stirred tank
3) Incubation for an assured assigned time based on RTD analysis
4) Automated pH and conductivity adjustment for FTC
5) Supply to FTC



▪RTD analysis based on mechanistic models for tubular and packed bed reactors.
▪ Narrow RTD is needed for an efficient reactor.

Residence time distribution (RTD)



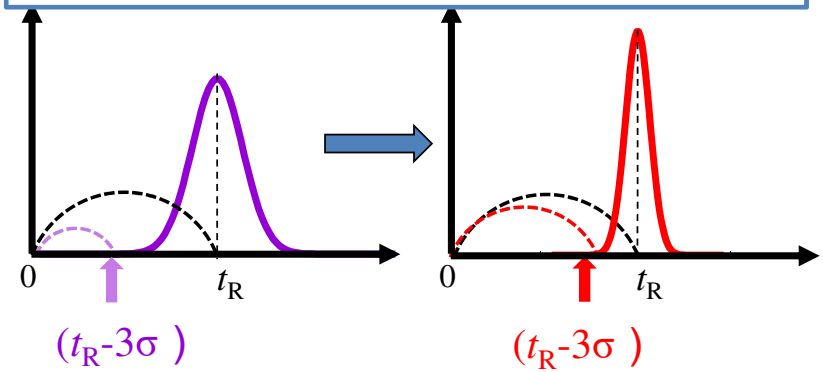


Relative RTD

$$t_R^* = \frac{(t_R - 3\sigma)}{t_R} \quad (3)$$

or

$$t_R^* = \frac{(t_R - 4\sigma)}{t_R} \quad (3')$$

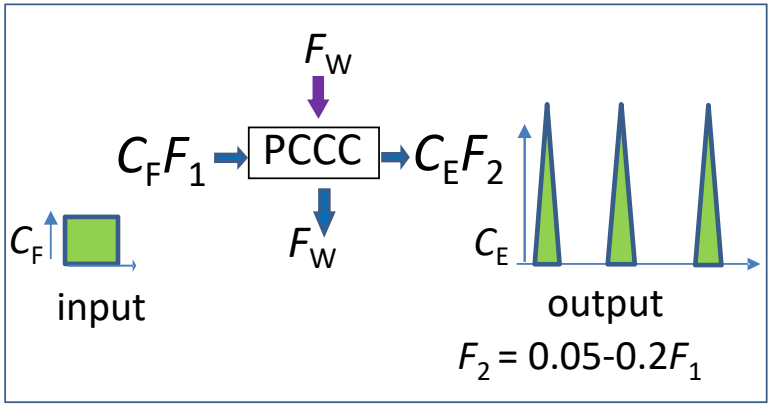


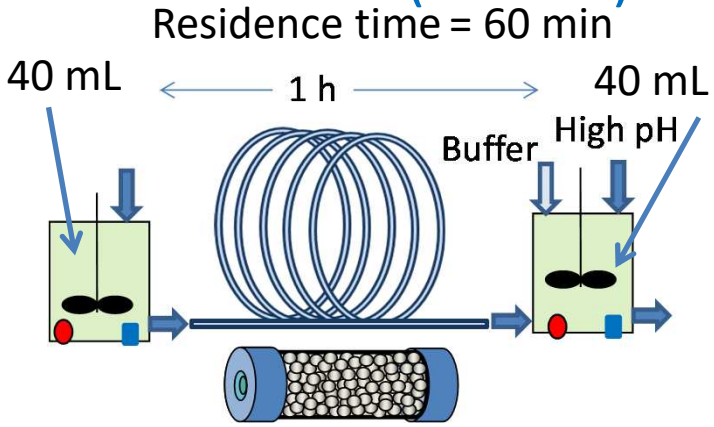
$t_R^* \Rightarrow 1$ narrrow RTD

[2] Practical limitations of continuous low pH virus inactivation by flow reactor

Scale-down study is always needed. Let consider the following case.

- Considering the pH electrode/the conductivity cell sizes, the volume of mixing tanks for adjusting pH/conductivity should be 30-50 mL.
- Namely, 60-100 mL + the volume of the reactor is needed.
- PCCC reduces the elution volume by a factor of 5-10. It takes 24 h to accumulate 100 mL PCCC fraction when the feed rate is 1000 mL/d.
  C_F = 2 g/L F_1= 1000 mL/d $F_2 = 0.1F_1$ = 100 mL/d
- It is practically useful to use the automated batch mixing vessel (tank) reactor, which can also work as a **CYCLE SURGE TANK** (N-mAb).

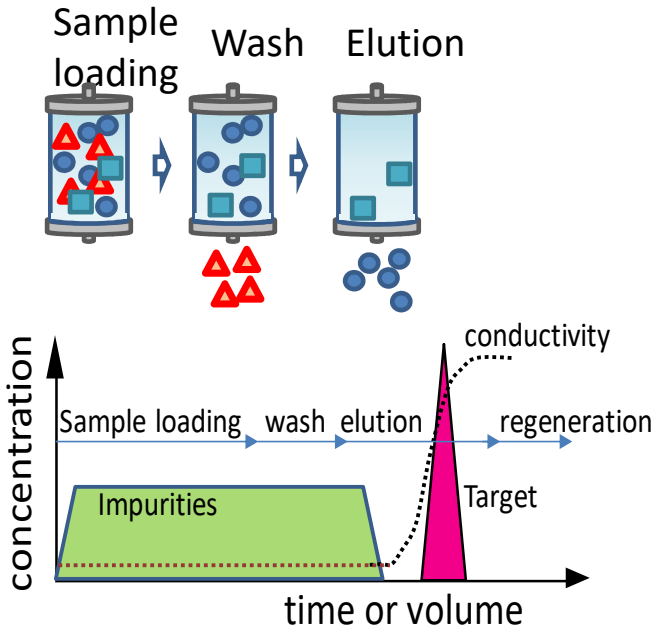




[3] Continuous polish chromatography Flow-through chromatography (FTC)

Bind/Elute chromatography

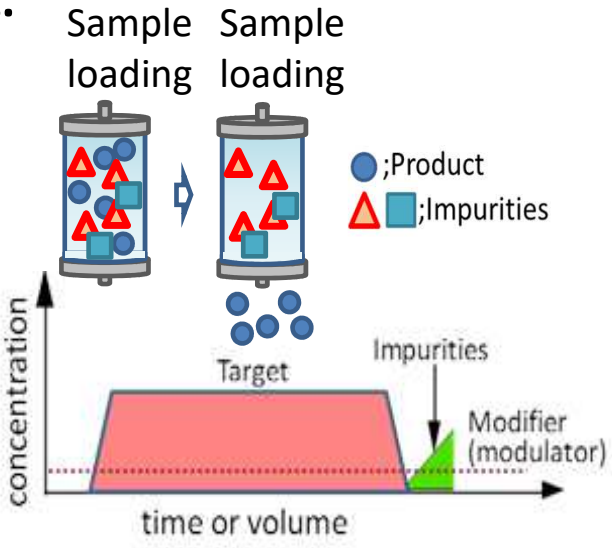
Target is first bound tightly to the column. Then, it is eluted by changing the mobile phase salt concentration and/or pH.



Flow through chromatography(FTC)

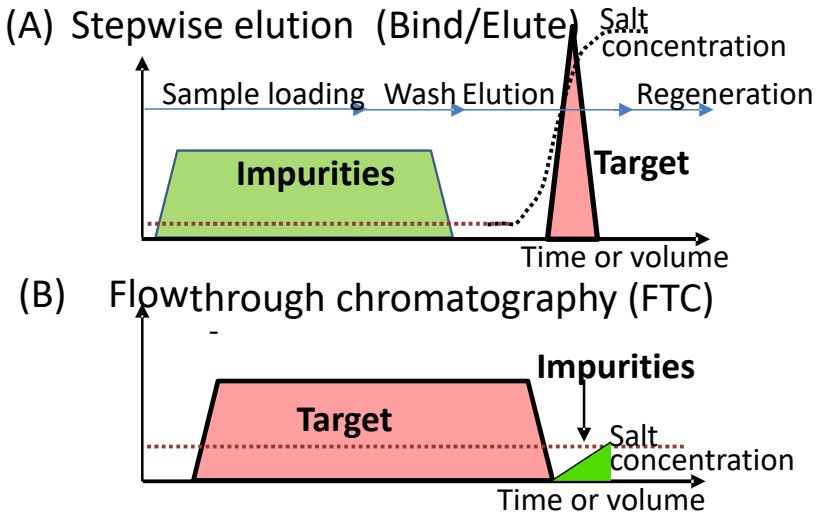
While impurities are bound to the column, the target protein is recovered without adsorption. As the amount of impurities for polish chromatography is small, FTC is a very efficient continuous method.

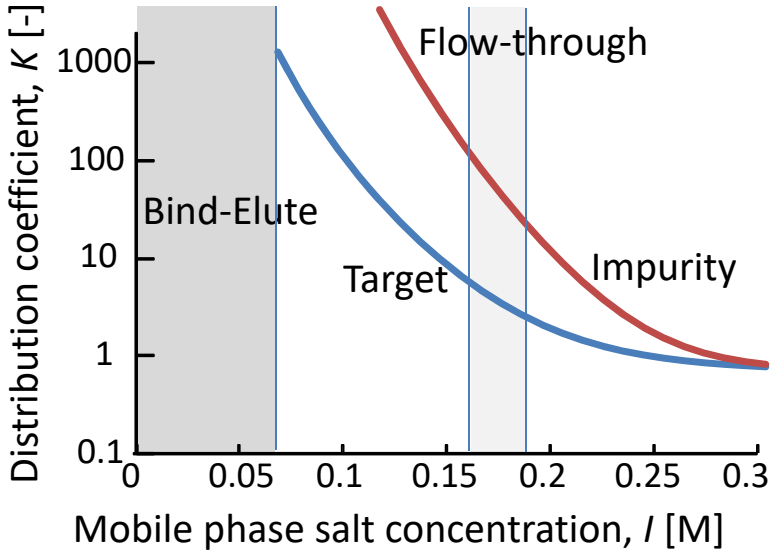
Choosing the right mobile phase is important.



[3] Continuous polish Flow-through chromatography (FTC)

FTC separation mechanism by Ion exchange chromatography (IEC) in terms of distribution coefficient *K*





- While the target molecule is eluted from the column continuously, the impurities are bound tightly.
- The sample loading should be stopped before the breakthrough of the impurities.
- Both the salt concentration (and pH) and the residence time affect the impurity breakthrough.

Accelerated method for designing flow-through chromatography of proteins, *J.Chem.Eng. Jpn.*, **53**, 206-213(2020)

Optimization of flow-through chromatography of proteins *J.Chem.Eng. Jpn.*, **53**, 214-221(2020)

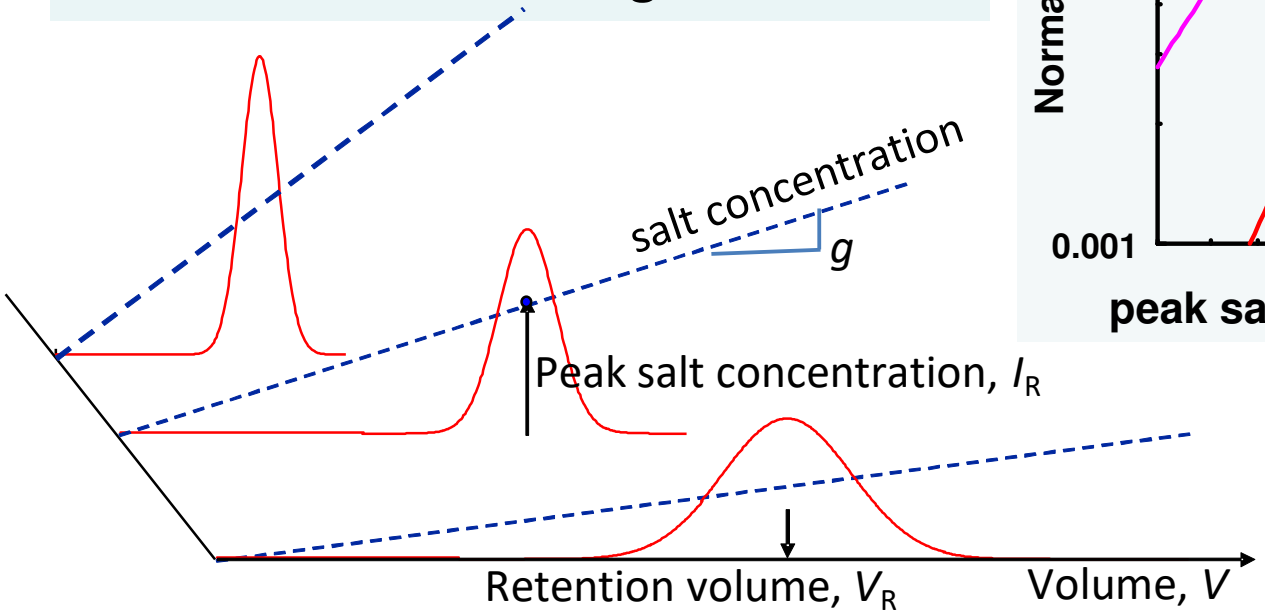
How to determine *K* as a function of *I* from linear gradient elution (LGE) data

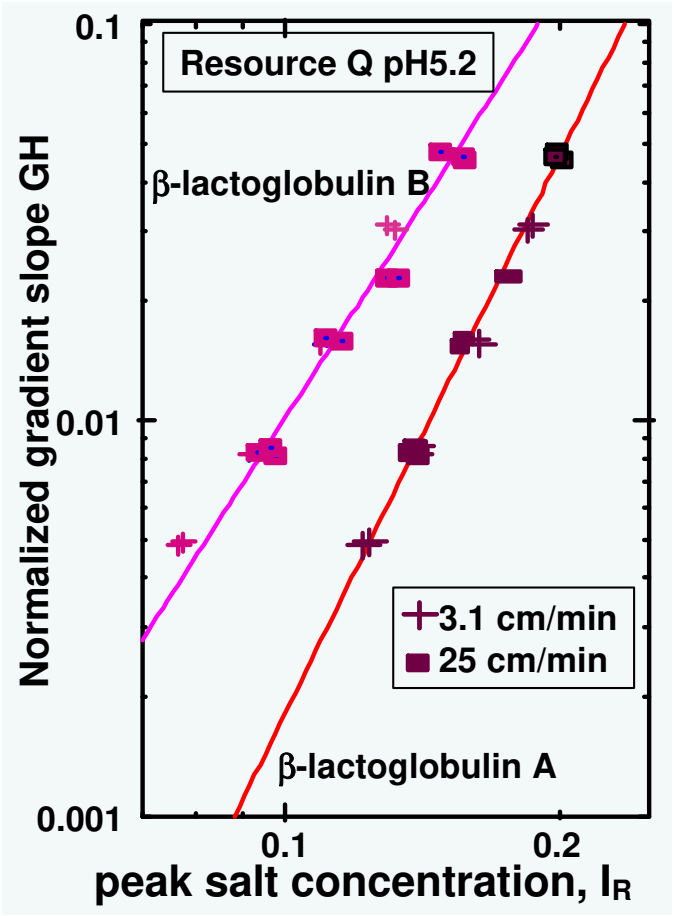
$GH = I^{(B+1)}/[A(B+1)] \rightarrow K = AI^{-B} + K_C$

$GH = g(V_t - V_o)$ V_t : column vol.

V_o : column void vol.

B :the number of biding sites





Flow-through chromatography – model simulation –

1) Linear gradient elution(LGE) experiments
   Prepare GH-I_R plots to determine A & B

2) K= f(I)=AI^{-B}+K_C for monomer and dimer

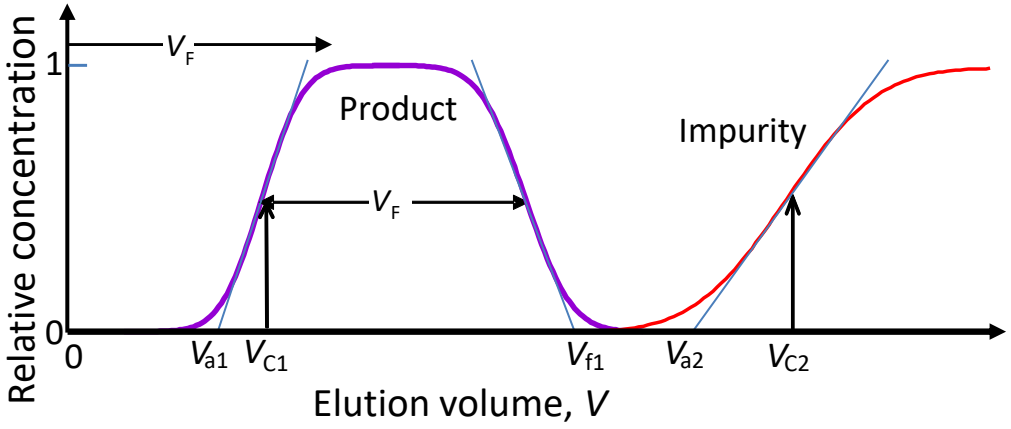
3) Single-zone spreading parameter model for elution curves C/C_0=f(N, K, V_F) where V_F is the sample feed volume.

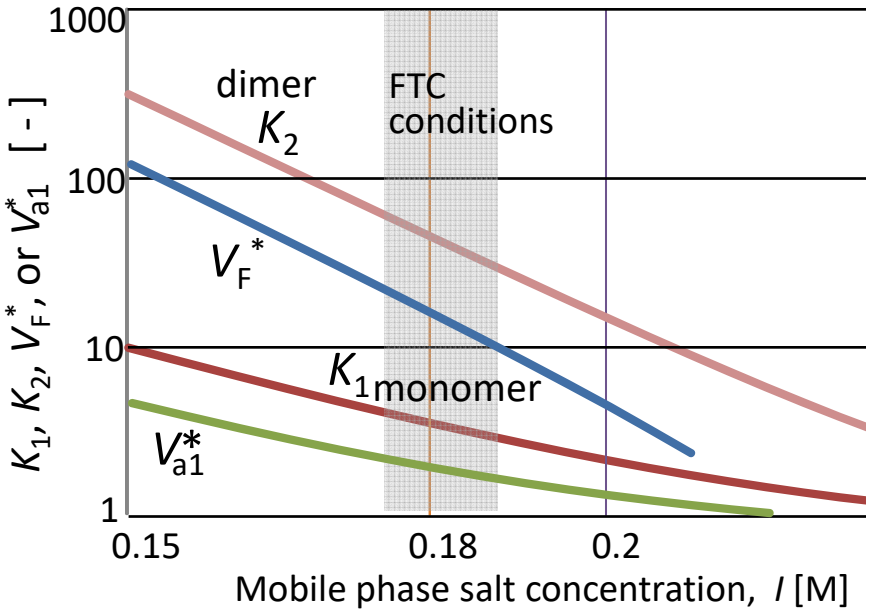
4)N is calculated by HETP equation, which includes the pore diffusivity, D_s and K.

5) BSA monomer-dimer separation on Q Sepharose HP

J.Chem.Eng. Jpn., **53**, 206-213, 214-221(2020)

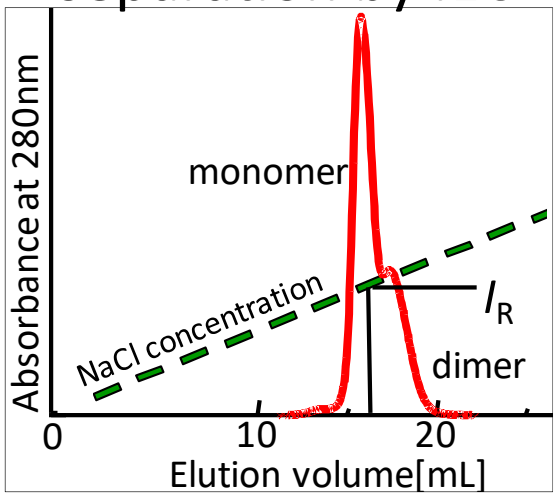
FTC process design method





J.Chem.Eng. Jpn., **53**, 206-213, 214-221(2020)

LGE of monomer separation by IEC



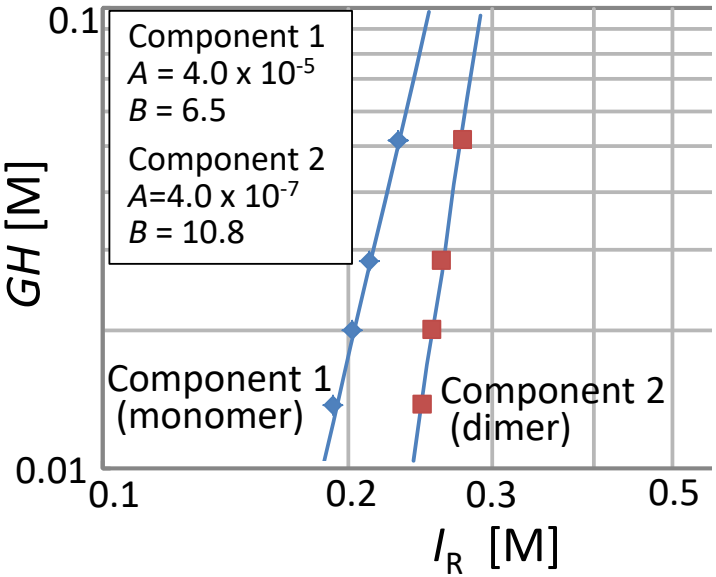
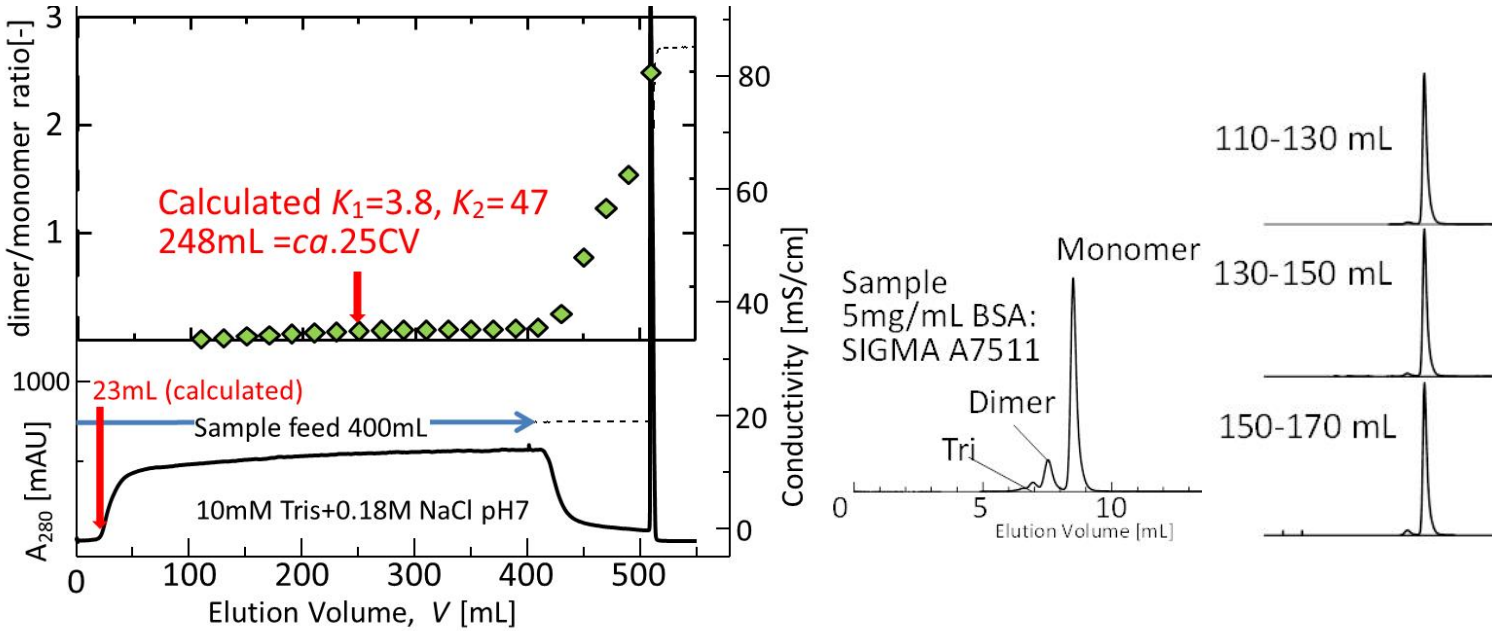


Table 1　Calculated and experimental breakthrough volumes of monomer and dimer

	Calc.	Calc.	Exp.	Calc.	Exp.
	K [–]	V_a^*[–]	V_a^*[–]	V_a[mL]	V_a [mL]
monomer	3.5	2.2	3.0	29.0	20
dimer	44.5	22.7	21.0	216	202



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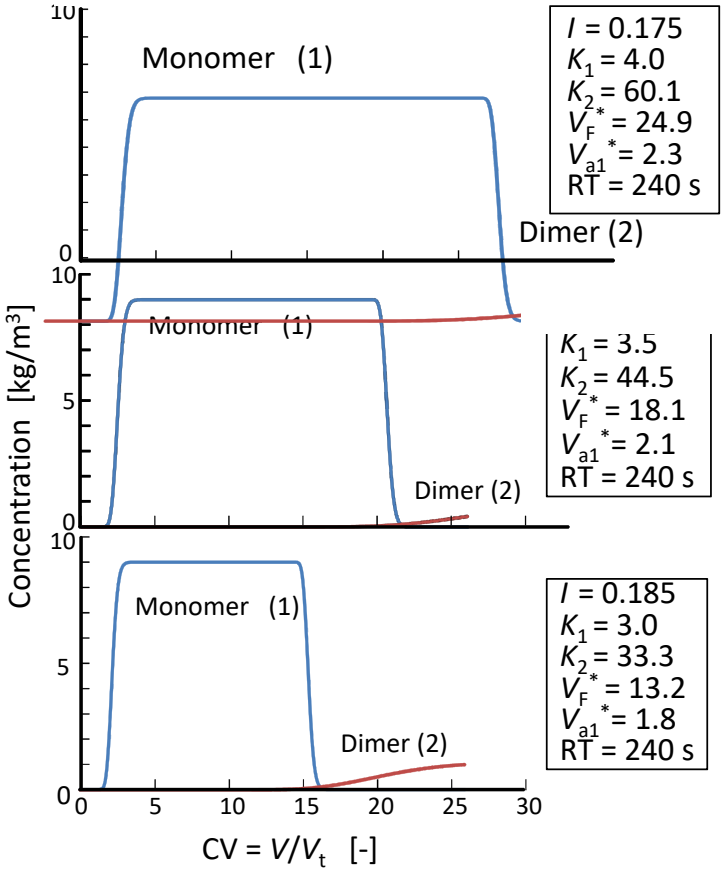
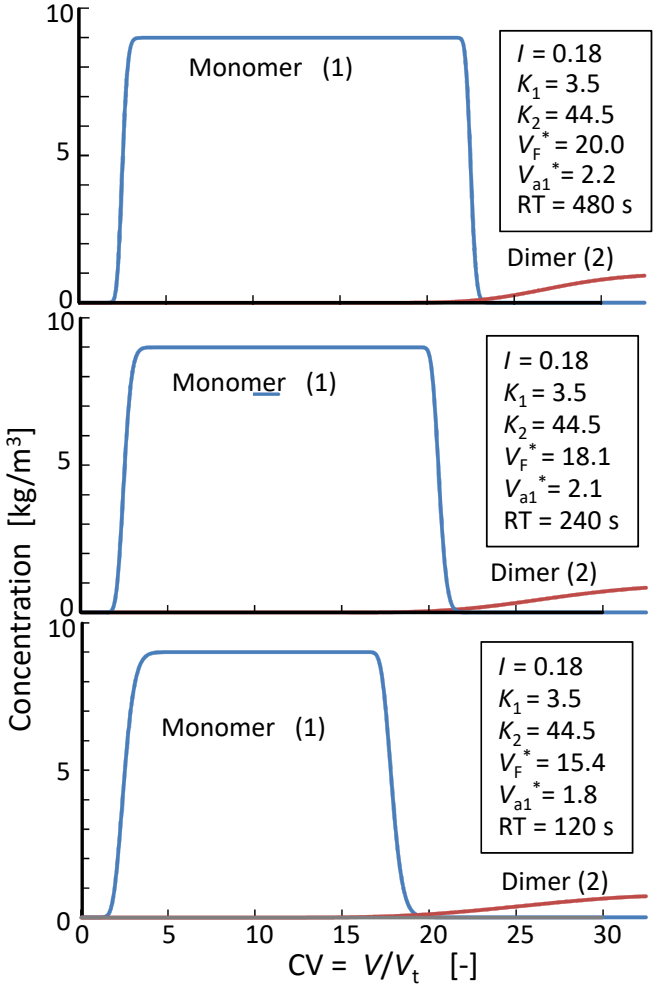
FTC process simulations

Effect of salt concentration, *I*

Very sensitive to a small change in *I*



Effect of residence time (RT)



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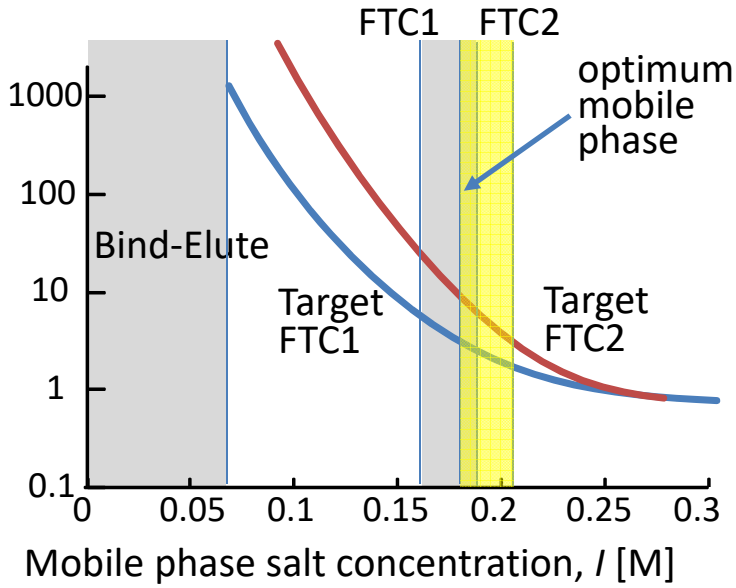
Choosing the right mobile phase salt concentration for FTC by ion exchange chromatography (IEC)



- Distribution coefficient *K vs*. mobile phase salt concentration *I* curve describes the binding strength.
- Tight binding when $K \gg 100$
- Weak binding and flow through when $K < 10$
- *K vs. I* curve is important and useful for choosing the right salt concentration *I*.
- Determining *K vs. I* curve is not easy. A method using Linear gradient elution data is recommended

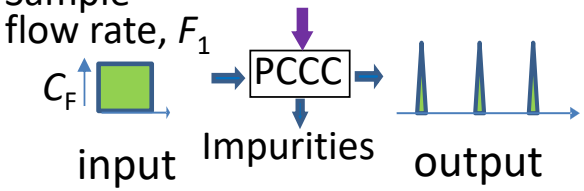
- When two different FTC steps are needed, the buffer exchange should be carried out after the 1st FTC.
- Two FTC columns can be connected for eliminating the buffer exchange.
- Searching for the optimum mobile phase is a difficult task.

[4] CDSP developed at MAB

Capture 2-column PCCC　Low pH virus inactivation (VI 1h)　FTC＋Virus filtration (VF)

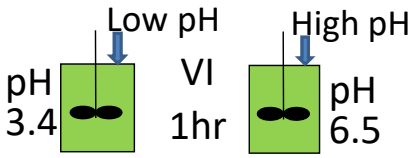
Wash buffer, elution buffer, CIP, buffer

Sample flow rate, F_1

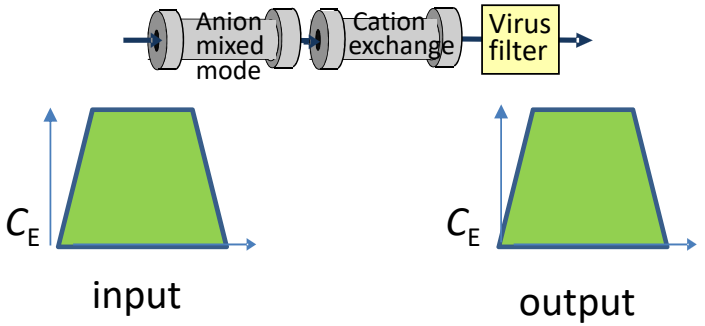


Residence time = 20-60 min

Batch reaction = 60 min

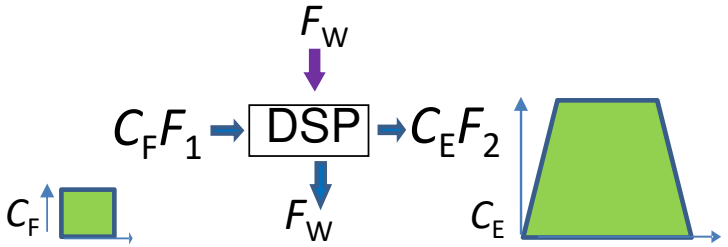


Sample flow rate $F_2 = 0.05\text{-}0.2F_1$



Residence time = 20-60 min

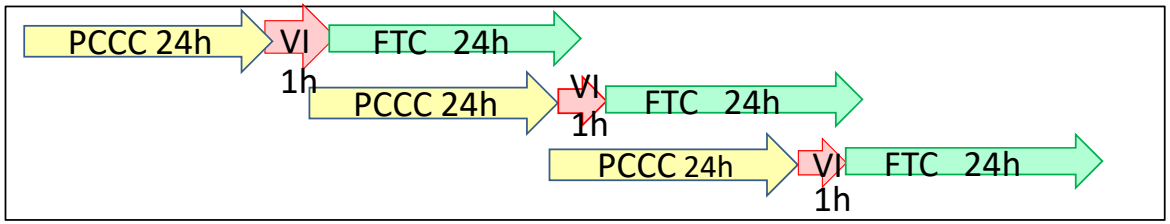
$C_F F_1 = C_E F_2$



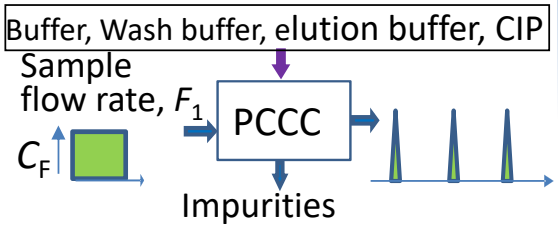
$F_2 = 0.05\text{-}0.2F_1$

[4] Concept of CDSP

Residence time
PCCC 24h (30-60min)
↓
VI (60-80 min)
↓
FTC(+VF) 24h (60 min)



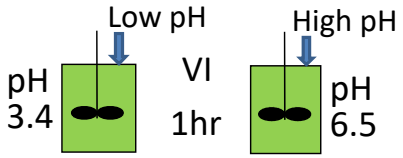
Capture 2-column PCCC



24 hr operation
(YMC Contichrom HPLC30)

Protein A chromatography
Kaneka or JSR

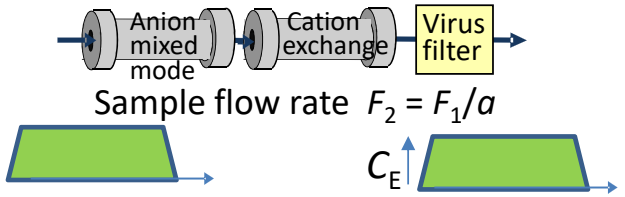
Low pH virus inactivation



Volume= $(24 \times F_1)/a$
$10 < a < 20$

Stirred tank reactor
Every 24 hs
The final solution was
filtered with 0.2 μm MF.

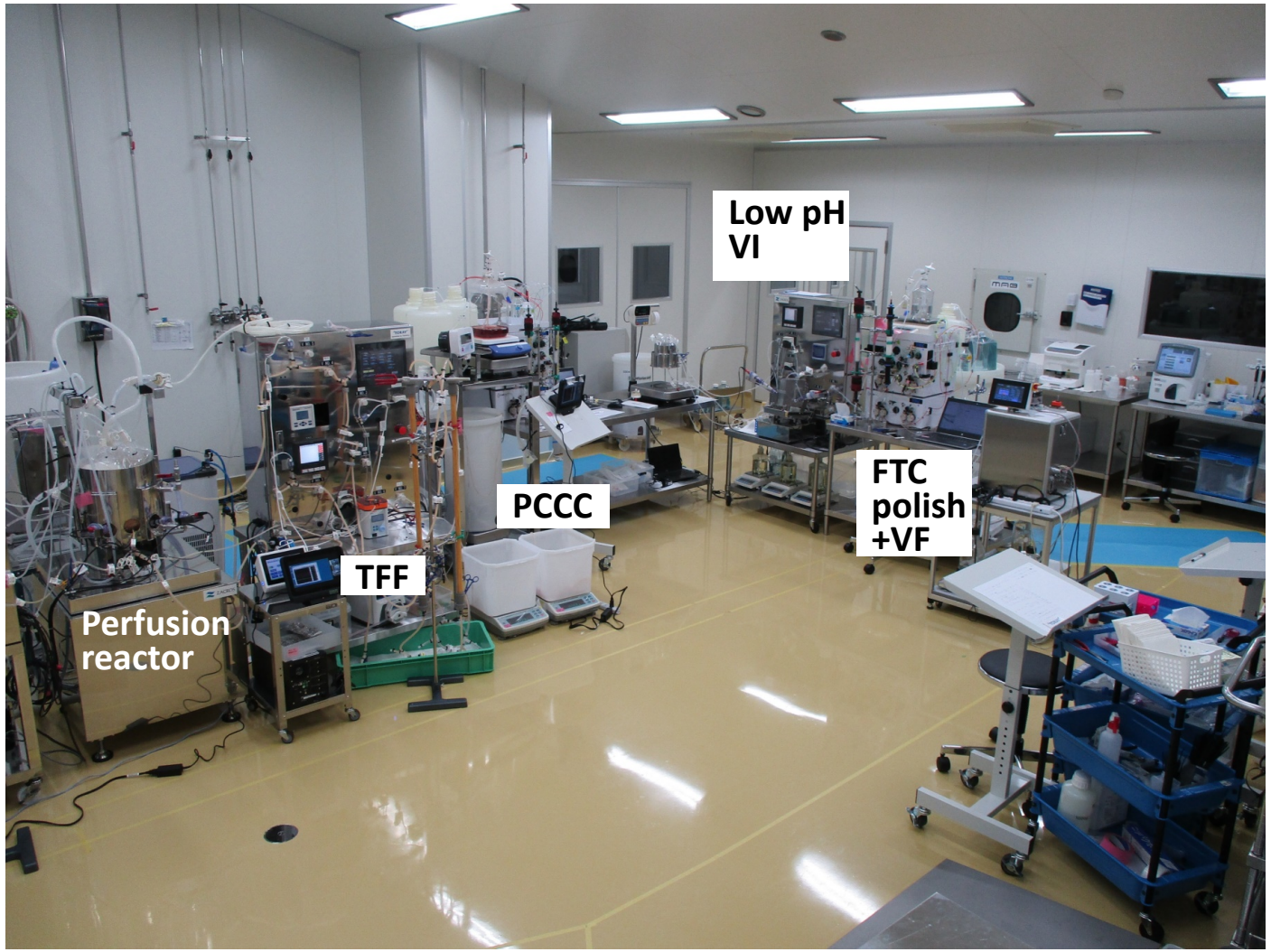
FTC＋Virus filtration (VF)



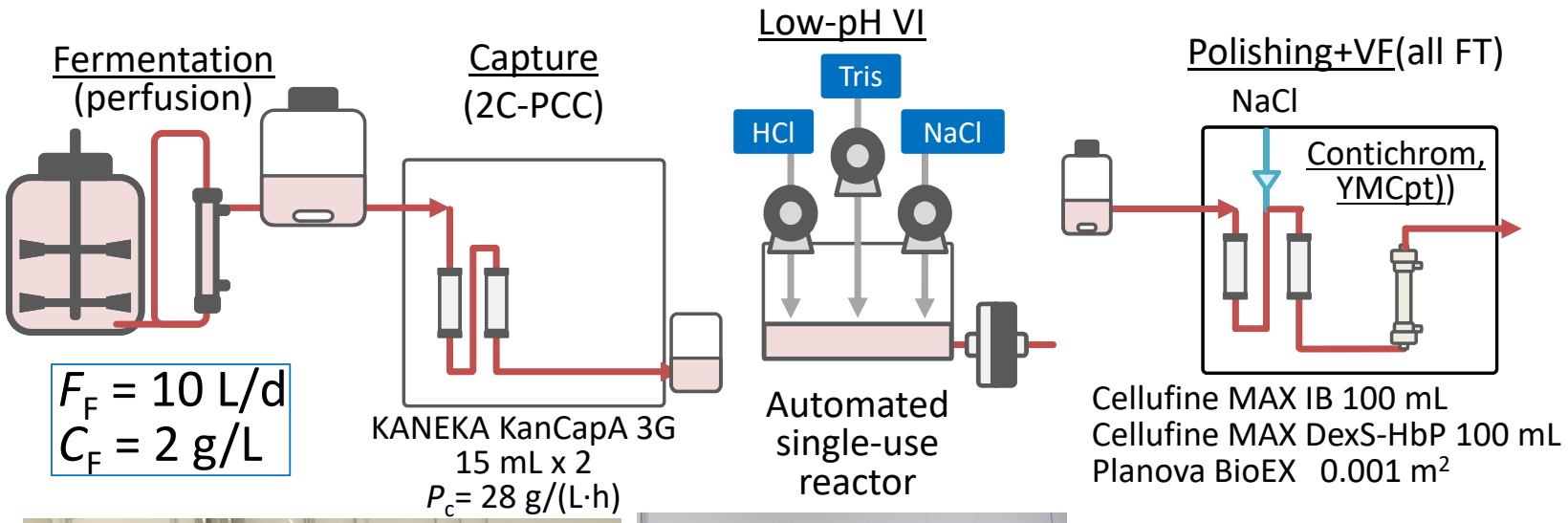
24h operation (AKTA Pure 150)

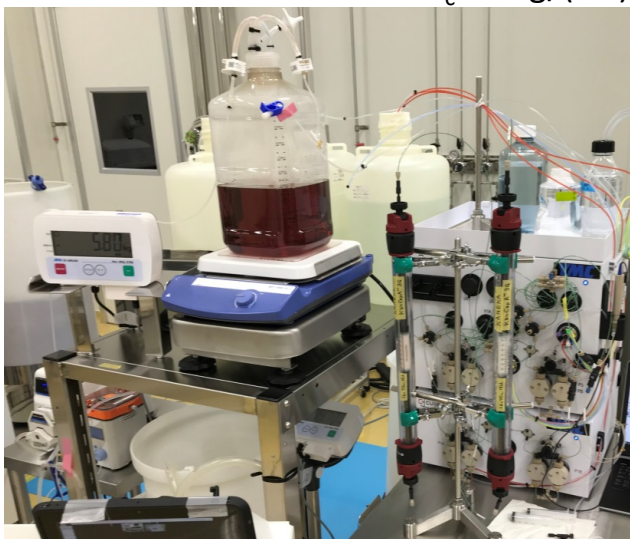
JNC；Mixed mode, Cation exchange
Asahi Kasei Meidal Planova

Run at MAB GMP facility in Kobe (connected to upstream)



Run at MAB GMP facility (connected to upstream)



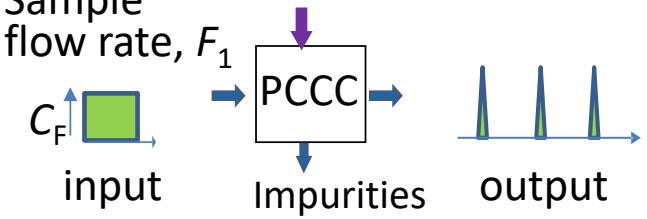


[4] CDSP developed at MAB

Capture 2-column PCCC　Low pH virus inactivation　FTC＋Virus filtration (VF)

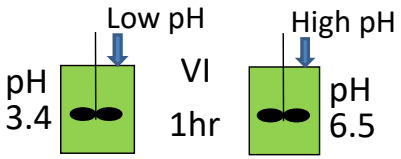
Wash buffer, elution buffer, CIP, buffer

Sample flow rate, F_1

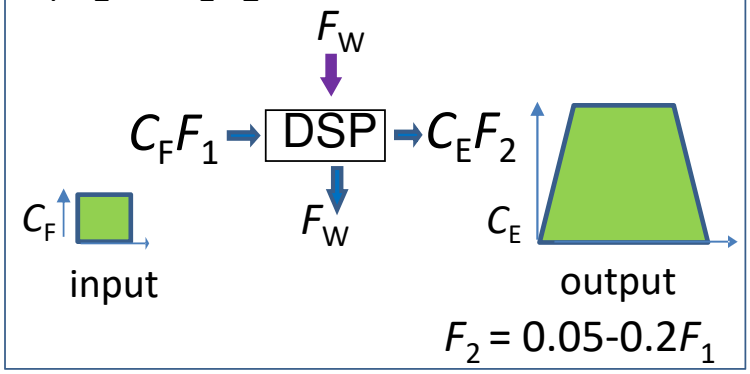


Residence time = 40-60 min

Batch reaction = 60 min

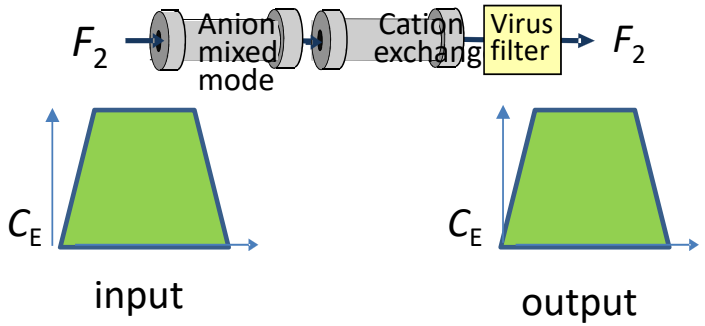


$C_F F_1 = C_E F_2$



$F_2 = 0.05\text{-}0.2F_1$

Sample flow rate $F_2 = 0.05\text{-}0.2F_1$



Residence time = 20-60 min

Summary

1. We have developed a continuous downstream process, which consists of 2-column PCCC, a batch VI reactor, and connected 2-column FTC (with VF). The batch VI reactor can work as a buffer or surge tank, and is useful for defining the batch size.
2. Six runs for five day operation were carried out with C_F=1- 3.2 g/L and F_F = 0.5-3.6 L/d. GMP run was carried out with C_F=2 g/L and F_F = 10 L/d, which was connected to the perfusion reactor.
3. The recovery was ca.80%>. The monomer purity by SEC was 95%. HCP clearance <10 ng/mg-IgG DNA clearance <1 pg/mg-IgG.
4. PCCC can reduce the buffer consumption up to 30-40%.
5. As PCCC output is not continuous but intermittent, and reduces the volume, the volumetric flow rate is reduced for the following processes. This is important to consider for scale-down studies, and to connect to the following virus inactivation reactor.

Summary (continued)

6. Mechanistic model based analysis is important for the continuous process characterization.

7. The process was not optimized. Further improvement of the process efficiency is possible.

8. Scale up to 10-50 L/d is straightforward with the current system.

9. It is also needed to develop the process performance criteria as the productivity itself is not sufficient for the assessment. Even the productivity of the whole process is still difficult to be defined.

10. Buffer consumption of FTC columns was not calculated as the single use was assumed. However, the repeated use with CIP and regeneration may be more environmentally friendly.

References (open access papers)

Prediction of the performance of capture chromatography processes of proteins and Its application to the repeated cyclic operation optimization.
Chyi-Shin Chen, Noriko Yoshimoto, Shuichi Yamamoto
J.Chem.Eng. Jpn., **53**, 689-697(2020) DOI:10.1252/jcej.20we116

A regressive approach to the design of continuous capture process with multi-column chromatography for monoclonal antibodies.
Chyi-Shin Chen, Fuminori Konoike, Noriko Yoshimoto, Shuichi Yamamoto
J.Chromatogr, A., **1658**, 462604(2021) DOI:10.1016/j.chroma.2021.462604

Linear flow-velocity gradient chromatography-An efficient method for increasing the process efficiency of batch and continuous capture chromatography of proteins.
Chyi-Shin Chen, Kosei Ando, Noriko Yoshimoto, Shuichi Yamamoto,
Biotechnology and Bioengineering., (2020) DOI: 10.1002/bit.27649

Accelerated method for designing flow-through chromatography of proteins
Sumiko Hasegawa, Chyi-Shin Chen, Noriko Yoshimoto, Shuichi Yamamoto
J.Chem.Eng. Jpn., **53**, 206-213(2020) DOI:10.1252/jcej.20we002

Optimization of flow-through chromatography of proteins
Sumiko Hasegawa, Chyi-Shin Chen, Noriko Yoshimoto, Shuichi Yamamoto
J.Chem.Eng. Jpn., **53**, 214-221(2020) DOI:10.1252/jcej.20we003

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